

Effect of Adding Sugar to Burley Tobacco on the Emission of Aldehydes in Mainstream Tobacco Smoke

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Objectives: Sugars in tobacco products enhance the taste and smoke characteristics of the blend. Sugars are often added to processed tobacco, particularly air-cured Burley tobacco leaves that contain virtually no sugars. The most commonly used sugars were systematically added to Burley tobacco to study the effect on aldehyde emissions in mainstream smoke. **Methods:** Two levels of sucrose, glucose, and fructose were added to Burley tobacco. Formaldehyde, acetaldehyde, acetone, acrolein, crotonaldehyde, propionaldehyde, and butanal in mainstream smoke were sampled on Carboxen 572 cartridges and determined by HPLC-DAD. **Results:** The addition of sugars to Burley tobacco resulted in an increase of the aldehydes acetaldehyde, acrolein, crotonaldehyde, propionaldehyde, and butanal in the mainstream tobacco smoke. This increase is specific, as much lower increases in tar, nicotine, and carbon monoxide levels were observed. The observed aldehyde level increases ranged from 5% to 40%. The increase was higher after the addition of fructose compared to sucrose and glucose. **Conclusions:** Sugars added to Burley tobacco increase the emissions of aldehydes, an important class of toxicants in tobacco smoke. Limiting sugars levels in processed tobacco may be an effective approach in tobacco product regulation to reduce the attractiveness of smoking, and the toxicants levels in cigarette smoke.

Key words: tobacco smoking; sucrose; glucose; fructose; aldehydes; toxicants in tobacco smoke

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Tobacco smoke contains more than 7000 chemicals;^{1,2} many of these chemicals are toxic, and more than 90 of these chemicals are identified as harmful and potentially harmful for smokers.^{3,4} Ingredients added during the manufacturing of tobacco products can be precursors of these harmful chemicals in tobacco smoke. Sugars are commonly used as one of the ingredients during

the manufacturing of cigarettes, mainly to enhance the taste and smoke characteristics of the blend.⁵

Studies report that when sugars such as glucose and sucrose are combusted or pyrolyzed, they yield organic compounds such as glyoxal, aldehydes, furfural and ketones which are hazardous when inhaled.⁶⁻⁸ Aldehydes and ketones formed include acetaldehyde, acrolein, crotonaldehyde, propional-

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dehyde, and methyl ethyl ketone (2-butanone).

The emission of aldehydes including formaldehyde, acetaldehyde, and acrolein from cigarette smoke has raised concerns over the health risk posed to both smokers and non-smokers. Formaldehyde, acetaldehyde and acrolein represent a class of volatile aldehydes which may cause a variety of hazardous effects when exposed to animals and humans. The US Environmental Protection Agency (EPA) classifies formaldehyde as a known human carcinogen,^{9,10} and acetaldehyde as a probable human carcinogen.^{11,12}

Formaldehyde and acetaldehyde are currently classified as Group 1 human carcinogen and Group 2B substance respectively by the International Agency for Research on Cancer (IARC).^{13,14} Both formaldehyde and acetaldehyde have been reported to cause nasal tumors in rats, at a concentration as low as 750ppm, where cytotoxic and neoplastic changes were observed.¹⁵⁻¹⁹ Aldehydes are known toxicants especially α , β -unsaturated aldehydes such as acrolein, crotonaldehyde. These compounds in cigarette smoke react with proteins and DNA, are cytotoxic to the cells and induce inflammatory responses.²⁰⁻²⁶ The DNA adducts of formaldehyde, acetaldehyde, acrolein, crotonaldehyde and propionaldehyde have been reported.²⁷⁻³³ Formaldehyde and acetaldehyde have been shown to cause harmful cardiovascular effects in animal studies.³⁴ Acrolein has been attributed to cause destabilization of atherosclerotic lesions, increase atherogenesis, induce dilated cardiomyopathy in animal toxicology risk models, and has been associated with smoking-related cardiovascular disease^{35,36}

Recognizing that aldehydes are an important class of toxicants, the WHO Study Group on Tobacco Product Regulation (TobReg) included 3 aldehydes (formaldehyde, acetaldehyde, and acrolein) in the list of toxicants recommended for mandated lowering³⁷ in tobacco product regulation. Although aldehydes have been identified in cigarette smoke since the 1960s,³⁸ the influence of ingredients, sugars in particular, added during the manufacturing process on the production of aldehydes has not been understood well.³⁹⁻⁴¹

To fill this research gap, we present the first independent study that systematically analyzes the effect of adding various sugars at 2 levels to untreated tobacco leaves. The present study aims to

analyze the effect of adding the widely-used sugars sucrose, glucose, and fructose on the emission levels of aldehydes in the smoke of make-your-own cigarettes. Sugars were added to cured Burley tobacco, because this tobacco type contains virtually no sugars,⁵ and therefore, the hypothesized increase in aldehydes will be most prominent. The influence of increasing levels of sugars added to cured Burley tobacco, its connection with aldehyde levels in mainstream smoke and implications for the use of sugar as tobacco additive will be discussed.

METHODS

The tobacco leave was analyzed for 3 different sugars: glucose, fructose, and sucrose. The mainstream tobacco smoke from make-your-own cigarettes was analyzed for tar, nicotine, carbon monoxide, and the aldehydes – formaldehyde acetaldehyde, acetone, acrolein, crotonaldehyde, propionaldehyde, and butanal (butyraldehyde).

Materials

Sugars added were fructose 99% (Accu standard Inc, New Haven, CT), and D-glucose 99% and sucrose 99.5% from Sigma-Aldrich (Germany). Tobacco leaf, Burley grade A, was obtained from Leave Only (USA). Standards of acetaldehyde, acrolein (1 mg/mL), and fructose (99.9%) were purchased from Sigma-Aldrich (Darmstadt, Germany). The other standards such as crotonaldehyde, butanal, propanal, and glucose (99.9%) were purchased from Accu standard Inc. (New Haven, CT). Acetonitrile (HPLC-grade, >99.9%), ethanol (>99.5%), phosphoric acid (85% solution in water), carbon disulphide (CS₂), 2,4-dinitrophenylhydrazine (DNPH, >99.5%) and carboxen-572 (CX-572) were from Sigma-Aldrich (Germany).

Preparation of cigarettes made from Burley tobacco leaves with and without sugar. Aqueous solutions of one type of sugar (from 50 mg/ml stock solutions of glucose, fructose or sucrose) were added to Burley tobacco (2 ml per gram of tobacco), resulting in 6 batches of cased tobacco differing in type and amount of sugar added. The treated tobacco was mixed for 24 hours at room temperature and shaken continuously to ensure uniform homogenization. The tobacco was dried in a conditioning closet for 2-3 days. The actual concentration of sugar was determined in the sugar

treated tobacco matrix. The amounts of sugar added ranged between 2% to 8%, which is the level commonly found in tobacco products such as cigarettes.⁴²⁻⁴⁴ The sugar treated tobacco was used to prepare cigarettes following the protocol described in ISO-15592. The adding of a sugar solution followed by mixing the resulting tobacco, is similar to the casing of tobacco, a common process in the manufacturing of cigarette.^{45,46} All cigarettes were prepared with filters (Gizeh, Golden Tip king-size).

Sugar analysis. Glucose, sucrose and fructose content of the Burley tobacco with and without these 3 sugars added, were determined in 6-fold. The sugar analysis was performed according to the method by Jansen et al⁴⁷ on a high-performance liquid chromatography (HPLC) system (Pro STAR, Varian, Middelburg, The Netherlands) equipped with an evaporative light scattering detector (ELSD) (ZAM 3000, Schambeck SFD GmbH, Bad Honnef, Germany) (80°C, gas flow 1.7 ml/min) using an analytical column MetaCarb 67C (Varian Assoc., Middelburg, The Netherlands). Briefly, the analysis is run on an isocratic elution with MilliQ water, with a flow rate of 0.5 mL per min, column temperature of 85°C, with injection volume of 20 µl and a total runtime of 20 min.

Mainstream Smoke Aldehyde Collection and Analysis

The cigarette smoke was generated on a single channel smoking in-house device using the ISO smoking regime.⁴⁸ Aldehydes were sampled on Carboxen 572 cartridges.⁴⁹ Each result is an average of 6 replicates with one cigarette smoked for each replicate.

Aldehyde determinations were conducted using a previously published method by Uchiyama et al.⁴⁹ The analysis was conducted on Shimadzu HPLC system (Shimadzu, Kyoto, Japan) equipped with a SPD M20A photo-diode array detector. The mobile phase used was MilliQ water acetonitrile (ACN) with an isocratic mode on a mixture of 1:1 (V/V) with a total run time of 40 minutes on a 1 mL per min flow rate. The column temperature was set at 30°C and injection volume of 10 µl was used according to the method which was slightly modified.⁴⁹ Each aldehyde result is an average of 6 replicates. The recovery for this method conducted on formaldehyde is between 103% and 113%; ac-

etaldehyde between 91% and 108% and acrolein from 94% to 102%.

Mainstream Smoke Tar, Nicotine, and Carbon Monoxide Analysis

The mainstream tobacco smoke levels of tar, nicotine and carbon monoxide were determined according to the ISO smoking regimen,⁵⁰⁻⁵³ using a semi-automated 20-channel, SM450 Cerulean linear smoking machine. Briefly, nicotine was analyzed using gas chromatograph (GC) coupled with a dual detector (FID and TCD); using analytical columns CP-WAX51 (25m x 0.25 mm x 0.2µm) for nicotine and porabond Q (25m x 0.32 x 5 µm) for water. The gas chromatograph was obtained from Shimadzu (Shimadzu GC2010-GC/02, Den Bosch, The Netherlands).

Data Analysis

The chromatograms were recorded and integrated with the Galaxie data system (Varian Inc) for sugars, Shimadzu Nexera Software for aldehydes and Varian system for nicotine. Data analysis was performed using Microsoft® Excel and GraphPad Prism 6.0h by Software MacKiev©.

RESULTS

Sugar Content

In Burley tobacco, sucrose, glucose, and fructose were not detected (LOD for glucose is 0.3 mg/g tobacco, for fructose 0.4 mg/g tobacco, and for sucrose 0.5 mg/g tobacco).⁴⁷ The final content of the different sugars added to Burley tobacco was 2.9% and 7.7% (w/w) for sucrose; 2.5% and 6.7% (w/w) for glucose and 3.7% and 5.4% (w/w) for fructose.

The Effects of the Addition of Sucrose, Fructose and Glucose to Burley Tobacco

The concentrations of the aldehydes in mainstream Burley tobacco smoke (µg per g tobacco) without added sugar were: formaldehyde 3.8, acetaldehyde 156.2, acetone 76.9, acrolein 6.7, propionaldehyde 9.2, crotonaldehyde 4.1, butanal 5.2, and total aldehydes 262. The relative changes in aldehyde levels upon sugar addition are reported below (aldehyde levels in mainstream smoke resulting from Burley with added sugars is available in Table 1 and Table 2 and its respective HPLC chromato-

Table 1
Aldehyde Levels in Mainstream Smoke (μg per g Tobacco), with Standard Deviation
(Average of 6 Cigarettes Replicates)

Burley	Formaldehyde (\pm SD)	% Change	Acetaldehyde (\pm SD)	% Change	Acetone (\pm SD)	% Change	Acrolein (\pm SD)	% Change
Without Sugar	3.8 (1.1)	n.a.	156.2 (21.6)	n.a.	76.3 (7.9)	n.a.	6.7 (0.7)	n.a.
Sucrose 2.96%	4.5 (0.8)	18.4	170.5 (23.2)	9.1	71.9 (12.6)	-5.7	9.5 (1.8)	40.7
Sucrose 7.74%	3.7 (1.1)	-2.0	193.6 (33.0)	23.9	88.3 (16.9)	15.6	11.6 (2.8)	72.6
Glucose 2.52%	4.6 (0.3)	23.0	161.0 (17.2)	3.0	76.3 (11.4)	0.0	7.9 (1.2)	17.0
Glucose 6.79%	3.2 (0.5)	-15.6	176.8 (23.3)	13.1	82.5 (12.7)	8.0	9.4 (1.6)	39.3
Fructose 3.73%	3.4 (0.6)	-9.8	195.0 (23.0)	24.8	91.4 (11.7)	19.7	9.1 (1.2)	35.5
Fructose 5.46%	2.0 (0.4)	-44.8	238.2 (29.7)	52.4	89.2 (11.4)	16.9	9.3 (2.3)	38.2
Average	---	-5.1	---	21.0	---	9.0	---	40.6

gram of aldehydes is available in Figures 1 and 2).

Sucrose. The observed relative changes in the emissions of all aldehydes are depicted in Figure 3. Generally, there is a statistically significant increase in the production of all aldehydes with increasing sucrose additions, except for formaldehyde. The

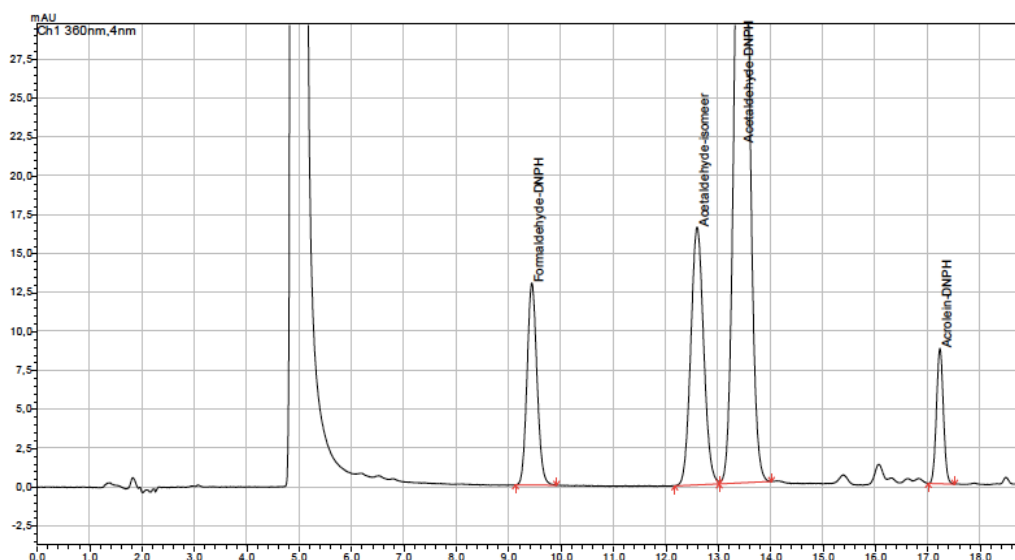
levels of acetaldehyde, acrolein, propionaldehyde crotonaldehyde, and butanal were found to increase by more than 20% with 7.7% sucrose added.

Glucose. The relative changes in the emissions of all aldehydes are depicted in Figure 4. Generally, the increase in all the aldehydes studied is rather

Table 2
Aldehyde Levels in Mainstream Smoke (μg per g Tobacco), with Standard Deviation
(Average of 6 Cigarettes Replicates)

Burley	Propionaldehyde (\pm SD)	% Change	Crotonaldehyde (\pm SD)	% Change	Butanal (\pm SD)	% Change	Total (\pm SD)	% Change
Without sugar	9.2 (1.6)	n.a.	4.1 (0.4)	n.a.	5.2 (1.1)	n.a.	262 (33)	n.a.
Sucrose 2.96%	11.1 (1.9)	21.1	5.8 (1.3)	40.3	6.5 (1.1)	23.2	280 (41)	7.0
Sucrose 7.74%	12.6 (2.7)	37.1	7.4 (1.4)	77.5	6.7 (1.2)	28.3	325 (58)	24
Glucose 2.52%	10.2 (1.2)	11.5	6.1 (1.1)	47.3	7.6 (0.6)	45.4	274 (32)	5
Glucose 6.79%	11.0 (1.6)	19.8	7.0 (1.2)	68.0	7.7 (1.0)	47.8	298 \pm 41	14
Fructose 3.73%	11.2 (1.6)	22.2	6.5 (0.9)	56.5	5.5 (0.8)	4.2	322 (39)	23
Fructose 5.46%	13.3 (2.1)	44.5	6.6 (2.0)	57.3	7.8 (1.0)	49.6	367 (46)	40
Average	---	26.0	---	57.8	---	33.1	---	19

Figure 1
Chromatogram of Aldehydes Standard Solution



like that of sucrose, except that the increases are relatively lower for acetaldehyde, acetone, acrolein and propionaldehyde. Both crotonaldehyde and butanal were found to have a significant increase of 45% to 68% with glucose added.

Fructose. The relative changes in the emissions of all aldehydes are depicted in Figure 5. Except

for formaldehyde and butanal, all 6 aldehydes demonstrated a statistically significant increase in mainstream tobacco smoke aldehyde levels after the addition of 3.7% (w/w) fructose. An additional increase was observed (except for acetone) with the addition of 5.4% (w/w) fructose, with the highest increase for butanal (50%), followed by acetalde-

Figure 2
Chromatogram of Mainstream Tobacco Smoke Extract

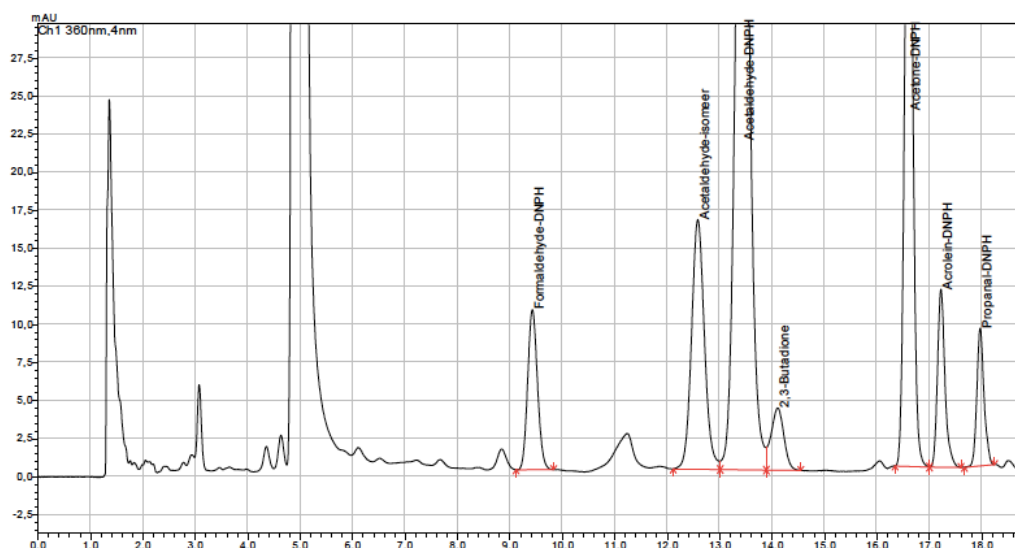
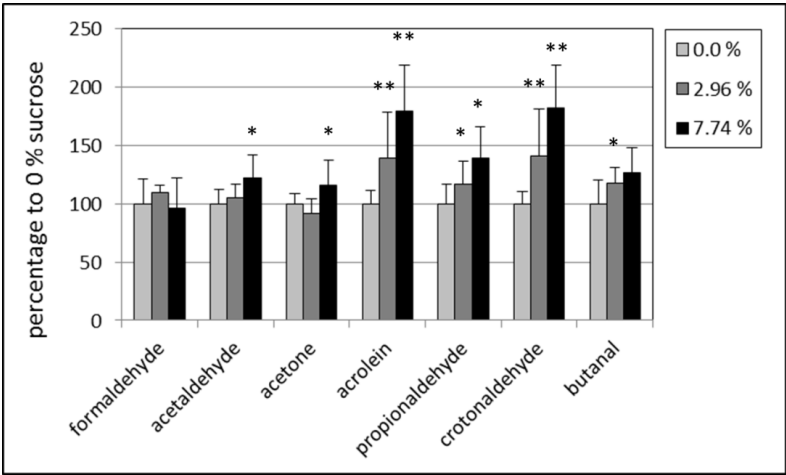


Figure 3
Relative Aldehyde Levels in Mainstream Tobacco Smoke of Burley Tobacco with 0%, 3.0%, and 7.7% of Added Sucrose



*p < .05; **p < .01

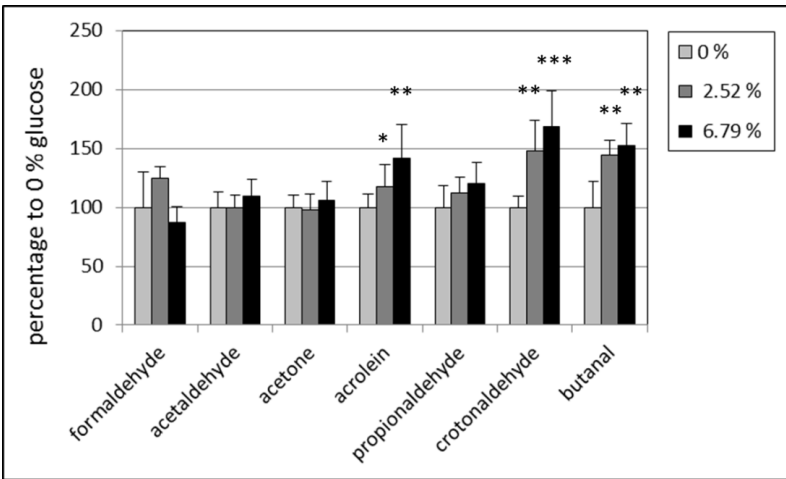
hyde (52%), and propionaldehyde (45%).

Total Aldehyde Levels

The results showed that, on average, the total al-

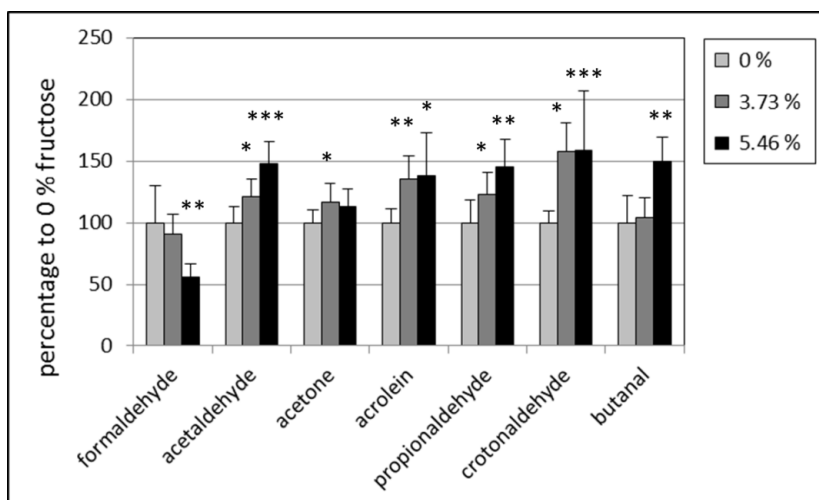
dehyde yield produced increased with 19% with addition of sugar, with the lowest increase of 5% (glucose 2.5%) and with the highest increase of 40% generated with the addition of fructose 5.4%. The highest overall total aldehyde yield increase

Figure 4
Relative Aldehyde Levels in Mainstream Tobacco Smoke of Burley Tobacco with 0%, 2.5%, and 6.8% of Added Glucose



*p < .05; **p < .01; ***p < .001

Figure 5
Relative Aldehyde Levels in Mainstream Tobacco Smoke of Burley Tobacco
with 0, 3.7%, and 5.5% of Added Fructose



*p < .05; **p < .01; ***p < .001

was found to be generated from the addition of fructose, followed by sucrose and glucose.

Tar, Nicotine, and Carbon Monoxide Analysis

To check whether the observed increases are selective for aldehydes, we also measured tar, nicotine, and carbon monoxide (TNCO) levels in mainstream smoke, because changes in the combustion process induced by sugars might have also resulted in changes in TNCO levels. As fructose is the most commonly used sugar additive in the production of cigarette (as high fructose corn syrup), we determined TNCO in mainstream tobacco smoke from Burley tobacco with and without 6% fructose. Variations of the measured tar, nicotine and carbon

monoxide levels of the tobacco with and without 6% added fructose in mainstream Burley tobacco smoke are statistically not significant with $p > .05$, refer to Table 3.

Discussion

There is accumulating evidence that aldehydes, one of the major groups of chemicals found in mainstream cigarette smoke, are related to the development of tobacco-related diseases.^{3,54} Also, aldehydes such as acetaldehyde are responsible for various pharmacological effects, including anxiogenic response when dealing with stress,⁵⁵⁻⁵⁷ and reinforcing self-administration of nicotine,^{58,59} thereby increasing the addictive effect of tobacco addiction.^{60,61}

Table 3
Tar, Nicotine, and Carbon Monoxide Emissions of Burley Tobacco with and without 6% Fructose Added (mg per g of Tobacco)

Tobacco	Total Particulate Matter \pm SD (mg/g)	Tar \pm SD (mg/g)	Nicotine \pm SD (mg/g)	CO \pm SD (mg/g)
Burley	24.6 \pm 3.0	21.0 \pm 2.0	2.8 \pm 0.2	21.6 \pm 0.8
Burley with 6% Fructose	29.4 \pm 2.9	24.7 \pm 2.3	2.7 \pm 0.2	23.9 \pm 1.4
% Difference	19.4	17.5	-3.1	10.5

For decades, there has been an on-going debate on the effects of added sugars and the level of aldehydes produced in mainstream tobacco smoke.^{62,63} The tobacco industry reports that formaldehyde levels are mainly increased in mainstream tobacco smoke.⁶⁴ In a review on the effects of sugars on aldehyde levels, sugars were reported to increase the level of formaldehyde, acetaldehyde, acetone, acrolein, and 2-furfural in tobacco smoke.⁷ Roemer⁶⁵ reported that formaldehyde, acrolein and 2-butanone increased in a statistically significant manner with sugar addition, but other authors only report an increase in formaldehyde levels.^{66,67} Hahn and Schaub also report a statistically significant increase in formaldehyde upon addition of 5% sucrose as compared to a reference tobacco blend consisting of 50% Virginia tobacco (flue-cured), 20% Burley tobacco (air-cured), 20% tobacco stems, and 10% Oriental tobacco (sun-cured).⁶⁴ It needs to be noted that Virginia tobacco contains high levels of natural sugars,⁶² which will make the relative increases in aldehyde levels upon adding additional sugar less prominent. Our study is the first non-tobacco industry work that investigates the effect of added sugars on aldehydes in mainstream tobacco smoke. We investigated the effect of adding different concentrations of sucrose, glucose, and fructose on the production of aldehydes in mainstream Burley tobacco smoke. Burley tobacco does not contain sugar or contains a minimal amount of up to 0.2% after air-curing.⁵ In general, we found that cigarettes with added sugar emit higher amounts of all aldehydes studied (acetaldehyde, acetone, acrolein, propionaldehyde, crotonaldehyde, and butanal), except for formaldehyde.

For acrolein and crotonaldehyde, the increase is rather prominent for all sugars, but levels off at higher fructose concentrations (see Figures 3-5, where the absolute levels together with their error bars and significances are depicted). The fact that we did not corroborate the reported increase in formaldehyde levels in several studies in the literature is likely because the method for aldehyde determination used in our study is not optimal for collection of formaldehyde, due to its highly volatile and hydrophilic nature. Some amount of formaldehyde may have been trapped in the Cambridge filter pad before the CX-572 cartridge.⁴⁹ Another published method on formaldehyde reported high variability in the reproducibility and repeatability.⁶⁸

Thus, it is too premature to derive any conclusion on the production of formaldehyde. Given the uncertainties in the analytical measurement on formaldehyde, further works is needed to optimize and refine the methods to understand its emission behavior.

Sugars such as sucrose, glucose, and fructose are among the ingredients added to tobacco during the manufacturing of cigarettes to enhance the flavour.^{5,69,70} The sugar concentrations in this study ranges between 3% to 8% (w/w), which is representative of typical sugar levels added to commercial cigarettes.^{65,69,71,72} The amount of added invert sugar (mixture of glucose and fructose) ranges between 3.3% and 5.2% in cigarettes manufactured for sale in countries such as The Netherlands,⁴³ Singapore,⁴² South Africa,⁷³ and Uruguay.⁴⁴

Among the 3 sugars added, the most pronounced increase in aldehyde levels was observed with fructose, where for the concentrations studied, the total aldehyde levels were found to increase 23% and 40%, respectively. The reactivity of glucose is less than fructose due to the formation of stable ring structures such as glucopyranose and glucofuranose, which inhibit its reactivity.⁷⁴ Fructose also forms both pyranose and furanose structures,⁷⁵ but exists to a larger extent in the open chain position than glucose.⁷⁶ As a result, fructose is more reactive than glucose due to the formation of glycation end-products as a result of the reaction of the aldehyde group of sugars with amino side chains in proteins.^{77,78} Therefore, it is not surprising that fructose shows a higher yield of several aldehydes during combustion of tobacco than glucose.

The increases in aldehyde levels are specific, as much smaller increases in tar, nicotine, and carbon monoxide levels were observed. Apparently, changes in the combustion process or other processes induced by sugars did not result in changes in TNCO levels. That the addition of sugar did not alter the nicotine produced in the mainstream cigarette smoke is consistent with earlier studies.⁷⁹⁻⁸¹ Because the nicotine level does not increase with adding sugar, in contrast to the aldehyde levels, the aldehydes-to-nicotine ratios are higher in cigarettes with added sugars. Thus, smokers who are addicted to nicotine will be exposed to more aldehydes, because they aim for a specific nicotine level. This emphasizes the need to normalize toxicant levels to

nicotine levels as measure of human exposure, as proposed by TobReg.³⁷

Future research should include the effect of adding sugar mixtures, a wider range of doses, and different tobacco types. We added only single sugars and not a mixture of sugars, which may be closer to the real smoking situation. The most common ingredient used as sugar ingredient is high fructose corn syrup (HFCS), with the percentage of fructose relatively higher (60% fructose and 40% glucose).

In addition, a limited range of doses has been studied, and it also would be interesting to see the effect of the cigarette production process on the results, such as the effect of filler density. Finally, it would be interesting to study other types of tobacco, most notably bright tobacco (Virginia), because it contains prominent levels of natural sugars. The amount of sugar present ranges from less than 0.2% in air-cured Burley varieties,⁵ to 10% in Oriental, and up to 25% in flue-cured Virginia leaves.⁶² Cigarette fillers are made up of a single type of tobacco leave such as Virginia or Burley or a mixture of diverse types such as Virginia with Burley and/or Oriental. Studying these tobacco types may help elucidate whether there are differences in aldehyde levels between equal levels of added sugar and naturally present sugar. On the other hand, Virginia tobacco and Burley tobacco are different in many other respects regarding their chemical composition, and many other components in tobacco leaves also may affect the composition of tobacco smoke.

Implications for tobacco regulation

Tobacco additives have become a major debate among policymakers in tobacco product regulation,⁴¹ due to their impact on making the cigarette smoke less harsh, and their balancing of taste; hence, additives make it more attractive to initiate and continue smoking. Ingredients or additives added in the production of cigarettes also may contribute to the addictive potential of tobacco use⁴¹ in addition to reducing harshness.^{82,83}

Sugars are common ingredients added during the manufacturing of tobacco products such as cigarettes. Sugars added during the tobacco manufacturing process promote acceptability by the smoker as they reduce the harsh taste of the smoke. This is due to the caramel flavors and acids generated from

sugars during the smoking process, an effect appreciated by smokers and experimenters. Our study shows that sugars added to Burley tobacco increase the emission of aldehydes, an important class of toxicants in tobacco smoke.

Cigarettes concentrated with sugars yield more than 20% higher amounts of acetaldehyde, acrolein, propionaldehyde, crotonaldehyde, and butanal (butyraldehyde). This raises concern that sugar as an additive lead to an increased health risk associated with cigarette smoking. Limiting the amount of the sugar content of cigarettes may be one of the possible approaches in tobacco regulation in reducing aldehydes generated from smoking. Among the 3 sugars studied, fructose caused the highest increase in total aldehydes produced in mainstream Burley tobacco smoke. This could be explained by the higher reactivity of fructose relative to glucose and sucrose.

As research independent of the tobacco industry, this paper adds to the existing knowledge and previously published reports that higher sugar content in tobacco leads to higher aldehyde yields in the smoke. Our results provide additional science-based arguments for regulators and policymakers in setting measures for regulating additives in the manufacturing of cigarette, in this case, low molecular weight sugars. The prohibition of sugar as an ingredient in the production of cigarettes was implemented in Canada in 2009,⁸⁴ which demonstrated that it is possible to manufacture cigarette without sugars.

Conflict of Interest Statement

The authors declare no conflict of interest.

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References

1. Rodgman A, Perfetti TA. *The Chemical Components of Tobacco and Tobacco Smoke*. New York, NY: CRC Press; 2013.
2. Tobacco Atlas. *The Tobacco Atlas*. 5th ed. Atlanta, GA: American Cancer Society; 2015. Available at: <http://www.tobaccoatlas.org/>. Accessed December 28, 2017.
3. Talhout R, Schulz T, Florek E, et al. Hazardous compounds in tobacco smoke. *Int J Environ Res Public Health*. 2011;8(2):613-628.
4. US Food and Drug Administration (FDA). *Harmful and Potentially Harmful Constituents in Tobacco Products and Tobacco Smoke*. [Docket No. FDA-2012-N-0143]. Washington, DC: Department of Health and Human Services, FDA; 2012. Available at: <https://www.fda.gov/TobaccoProducts/GuidanceComplianceRegulatoryInformation/ucm297786.htm>. Accessed December 28, 2017.
5. Leffingwell JC. Basic chemical constituents of tobacco leaf and differences among tobacco types. In Layten Davis D, Nielson MT, eds. *Tobacco, Production, Chemistry and Technology*. Oxford, UK: Wiley-Blackwell; 1999.
6. Zhou XW, Nolte MW, Mayes HB, et al. Experimental and mechanistic modeling of fast pyrolysis of neat glucose-based carbohydrates. 1. Experiments and development of a detailed mechanistic model. *Ind Eng Chem Res*. 2014;53(34):13274-13289.
7. Talhout R, Opperhuizen A, van Amsterdam JG. Sugars as tobacco ingredient: effects on mainstream smoke composition. *Food Chem Toxicol*. 2006;44(11):1789-1798.
8. Yaylayan VA. Classification of the Maillard reaction: a conceptual approach. *Trends Food Sci Technol*. 1997;8:13-18.
9. Agency for Toxic Substances and Disease Registry (ATSDR). *Toxic Substances Portal - Formaldehyde*. Atlanta, GA: ATSDR; 2016. Available at: <https://www.atsdr.cdc.gov/toxfaqs/faq.asp?id=219&tid=39>. Accessed August 24, 2017.
10. Consumer Product Safety Commission (CPSC). An Update on Formaldehyde. 2016. Available at: <https://www.cpsc.gov/PageFiles/121919/An-Update-On-Formaldehyde-725.pdf>. Accessed August 24, 2017.
11. US Environmental Protection Agency (EPA). Chemical Assessment Summary : Formaldehyde. 2014. Available at: <https://www.epa.gov/iris/supporting-documents-formaldehyde>. Accessed August 24, 2017.
12. US Environmental Protection Agency (EPA). Toxicological Review of Formaldehyde-Inhalation Assessment: in Support of Summary Information on the Integrated Risk Information System. 2010. Available at: <https://www.gpo.gov/fdsys/pkg/FR-2010-06-02/html/2010-13097.htm>. Accessed January 1, 2018.
13. International Agency for Research on Cancer (IARC). Formaldehyde 2012. 2012. Available at: http://monographs.iarc.fr/ENG/Classification/latest_classif.php. Accessed January 1, 2018.
14. International Agency for Research on Cancer (IARC). Acetaldehyde 1999. 1999. Available at: http://monographs.iarc.fr/ENG/Classification/latest_classif.php. Accessed January 1, 2018.
15. Woutersen RA, Appelman LM, Feron VJ, Van der Heijden CA. Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study: interim results after 15 months. *Toxicology*. 1984;31(2):123-133.
16. Woutersen RA, Appelman LM, Van Garderen-Hoetmer A, Feron VJ. Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. *Toxicology*. 1986;41(2):213-231.
17. Wilmer JW, Woutersen RA, Appelman LM, et al. Subacute (4-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. *J Appl Toxicol*. 1987;7(1):15-16.
18. Wilmer JW, Woutersen RA, Appelman LM, et al. Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. *Toxicol Lett*. 1989;47(3):287-293.
19. Feron VJ, Arts JH, Kuper CF, et al. Health risks associated with inhaled nasal toxicants. *Crit Rev Toxicol*. 2001;31(3):313-347.
20. Song MK, Lee HS, Ryu JC. Integrated analysis of microRNA and mRNA expression profiles highlights aldehyde-induced inflammatory responses in cells relevant for lung toxicity. *Toxicology*. 2015;334:111-121.
21. O'Brien E, Spiess PC, Habibovic A, et al. Inhalation of the reactive aldehyde acrolein promotes antigen sensitization to ovalbumin and enhances neutrophilic inflammation. *J Immunotoxicol*. 2015;1-7.
22. Cheah NP, Pennings JL, Vermeulen JP, et al. In vitro effects of low-level aldehyde exposures on human umbilical vein endothelial cells. *Toxicol Res*. 2015;4:1250-1259.
23. Tamura M, Ito H, Matsui H, Hyodo I. Acetaldehyde is an oxidative stressor for gastric epithelial cells. *J Clin Biochem Nutr*. 2014;55(1):26-31.
24. Song MK, Choi HS, Lee HS, Ryu JC. Transcriptome profile analysis of saturated aliphatic aldehydes reveals carbon number-specific molecules involved in pulmonary toxicity. *Chem Res Toxicol*. 2014;27(8):1362-1370.
25. Pei ZH, Zhuang ZQ, Sang HF, et al. Alpha, beta-unsaturated aldehyde crotonaldehyde triggers cardiomyocyte contractile dysfunction: role of TRPV1 and mitochondrial function. *Pharmacol Res*. 2014;82:40-50.
26. Cheah NP, Pennings JL, Vermeulen JP, et al. In vitro effects of aldehydes present in tobacco smoke on gene expression in human lung alveolar epithelial cells. *Toxicol In Vitro*. 2013;27(3):1072-1081.
27. Chen HJ. Analysis of DNA adducts in human samples: acrolein-derived exocyclic DNA adducts as an example. *Molecular Nutrition & Food Research*. 2011;55(9):1391-1400.
28. Lee HW, Wang HT, Weng MW, et al. Acrolein- and 4-Aminobiphenyl-DNA adducts in human bladder mucosa and tumor tissue and their mutagenicity in human urothelial cells. *Oncotarget*. 2014;5(11):3526-3540.
29. Brooks PJ, Zakhari S. Acetaldehyde and the genome: beyond nuclear DNA adducts and carcinogenesis. *Environ Mol Mutagen*. 2014;55(2):77-91.

30. Bessette EE, Spivack SD, Goodenough AK, et al. Identification of carcinogen DNA adducts in human saliva by linear quadrupole ion trap/multistage tandem mass spectrometry. *Chem Res Toxicol*. 2010;23(7):1234-1244.
31. Wang M, Cheng G, Balbo S, et al. Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and nonsmokers. *Cancer Res*. 2009;69(18):7170-7174.
32. Zhang S, Villalta PW, Wang M, Hecht SS. Detection and quantitation of acrolein-derived 1,N2-propanodeoxyguanosine adducts in human lung by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Chem Res Toxicol*. 2007;20(4):565-571.
33. Islam BU, Moinuddin, Mahmood R, Ali A. Genotoxicity and immunogenicity of crotonaldehyde modified human DNA. *Int J Biol Macromol*. 2014;65:471-478.
34. Bhatnagar A. E-Cigarettes and cardiovascular disease risk: evaluation of evidence, policy implications, and recommendations. *Curr Cardiovasc Risk Rep*. 2016;10(24):1-10.
35. Haussmann HJ. Use of hazard indices for a theoretical evaluation of cigarette smoke composition. *Chem Res Toxicol*. 2012;25(4):794-810.
36. DeJarnett N, Conklin DJ, Riggs DW, et al. Acrolein exposure is associated with increased cardiovascular disease risk. *J Am Heart Assoc*. 2014;3(4).
37. Burns DM, Dybing E, Gray N, et al. Mandated lowering of toxicants in cigarette smoke: a description of the World Health Organization TobReg proposal. *Tob Control*. 2008;17(2):132-141.
38. US Public Health Service (USPHS). *Smoking and Health: Report of the Advisory Committee to the Surgeon General of the Public Health Service*. Washington, DC: USPHS; 1964. Available at: <https://profiles.nlm.nih.gov/NN/B/B/M/Q/>. Accessed January 1, 2018.
39. World Health Organization. The scientific basis of tobacco product regulation. *World Health Organization technical report series 951*. 2008:1-277. Available at: http://www.who.int/tobacco/global_interaction/tobreg/publications/9789241209519.pdf. Accessed January 6, 2018.
40. Bos MJP, Hernandez L, Mennes C W, et al. Risk assessment of tobacco additives and chemicals in cigarette smoke. 2012. Available at: <http://www.rivm.nl/bibliotheek/rapporten/340031001.html>. Accessed August 25, 2017.
41. Ferreira CG, Silveira D, Hatsukami DK, et al. The effect of tobacco additives on smoking initiation and maintenance. *Cadernos de saude publica*. 2015;31(2):223-225.
42. Philip Morris International. What's in Our Products? Singapore. Available at: <https://www.pmi.com/our-business/about-us/products/how-cigarettes-are-made>. 2012. Accessed January 1, 2018.
43. Philip Morris International. What's in our Products: Netherlands. Available at: <https://www.pmi.com/our-business/about-us/products/how-cigarettes-are-made>. 2014. Accessed January 1, 2018.
44. Philip Morris International. What's in Our Products? Japan. Available at <https://www.pmi.com/our-business/about-us/products/how-cigarettes-are-made>. 2013. Accessed January 1, 2018.
45. Leffingwell JC, Young HJ, Bernasek E. Tobacco flavoring for smoking products. Winston-Salem, NC: R J Reynolds Company; 1972,. Available at: <http://www.leffingwell.com/download/TobaccoFlavorBook.pdf>. Accessed January 6, 2018.
46. European Commission. Addictiveness and Attractiveness of Tobacco Additives. Available at: https://ec.europa.eu/health/scientific_committees/opinions_layman/tobacco/en/l-3/2.htm. Accessed September 9, 2017.
47. Jansen E, Cremers J, Borst S, Talhout R. Simple determination of sugars in cigarettes. *Analytical & Bioanalytical Techniques* 2014;5(6):1-3.
48. International Organization for Standardization. *Routine Analytical Cigarette-smoking Machine -- Definitions and Standard Conditions*. ISO3308:2012. Geneva, Switzerland: International Organization for Standardization; 2012.
49. Uchiyama S, Tomizawa T, Inaba Y, Kunugita N. Simultaneous determination of volatile organic compounds and carbonyls in mainstream cigarette smoke using a sorbent cartridge followed by two-step elution. *J Chromatogr A*. 2013;1314:31-37.
50. International Organization for Standardization. *Cigarettes - Determination of Nicotine in Smoke Condensates - Gas Chromatographic Method*. ISO10315:2013. Geneva, Switzerland: International Organization for Standardization; 2013.
51. International Organization for Standardization. *Cigarettes - Determination of Total and Nicotine-free Dry Particulate Matter Using a Routine Analytical Smoking Machine*. ISO4387:2000. Geneva, Switzerland: International Organization for Standardization; 2000.
52. International Organization for Standardization. *Cigarettes -- Determination of Water in Smoke Condensates -- Part 1: Gas-chromatographic Method*. ISO10362-1:1999. Geneva, Switzerland: International Organization for Standardization; 2010:1-7.
53. International Organization for Standardization. *Cigarettes - Determination of Carbon Monoxide in the Vapour Phase of Cigarette Smoke -- NDIR Method*. ISO8454:2007. Geneva, Switzerland: International Organization for Standardization; 2010:1-7.
54. Fowles J, Dybing E. Application of toxicological risk assessment principles to the chemical constituents of cigarette smoke. *Tob Control*. 2003;12(4):424-430.
55. Plescia F, Brancato A, Venniro M, et al. Acetaldehyde self-administration by a two-bottle choice paradigm: consequences on emotional reactivity, spatial learning, and memory. *Alcohol*. 2015;49(2):139-148.
56. Denoble VJ, Mele PC. Behavioral effects of termination of chronically administered nicotine- acetaldehyde combinations on lever pressing maintained under a fixed ratio schedule of food presentation in rats. Available at: <http://www.addictionincorporated.com/wordpress/wp-content/uploads/10-05-83.pdf>. Accessed February 12, 2016.
57. DeNoble VJ, Dragan VP, Carron L. Behavioral effects of intraventricularly administered (-)-nicotine on fixed ratio schedules of food presentation in rats. *Psychopharmacology (Berl)*. 1982;77(4):317-321.
58. Peana AT, Muggironi G, Diana M. Acetaldehyde-reinforcing effects: a study on oral self-administration behavior. *Front Psychiatry*. 2010;1:23.
59. Melis M, Diana M, Enrico P, et al. Ethanol and acetaldehyde action on central dopamine systems: mechanisms, modulation, and relationship to stress. *Alcohol*.

- 2009;43(7):531-539.
60. Cacace S, Plescia F, Barberi I, Cannizzaro C. Acetaldehyde oral self-administration: evidence from the operant-conflict paradigm. *Alcohol Clin Exp Res*. 2012;36(7):1278-1287.
61. Belluzzi JD, Wang R, Leslie FM. Acetaldehyde enhances acquisition of nicotine self-administration in adolescent rats. *Neuropsychopharmacology*. 2005;30(4):705-712.
62. Cahours X, Verron T, Purkis S. Effect of sugar content on acetaldehyde yield in cigarette smoke. *Beiträge zur Tabakforschung International/Contributions to Tobacco Research*. 2012;25(2).
63. Seeman JI, Laffoon SW, Kassman AJ. Evaluation of relationships between mainstream smoke acetaldehyde and "tar" and carbon monoxide yields in tobacco smoke and reducing sugars in tobacco blends of U.S. commercial cigarettes. *Inhal Toxicol*. 2003;15(4):373-395.
64. Hahn J, Schaub J. Influence of tobacco additives on the chemical composition of mainstream smoke. *Beiträge zur Tabakforschung International/Contributions to Tobacco Research*. 2010;24(3):100.
65. Roemer E, Schorp MK, Piade JJ, et al. Scientific assessment of the use of sugars as cigarette tobacco ingredients: a review of published and other publicly available studies. *Crit Rev Toxicol*. 2012;42(3):244-278.
66. Klus H, Scherer G, Muller L. Influence of additives on cigarette related health risks *Beiträge zur Tabakforschung International/Contributions to Tobacco Research*. 2012;25(3):412-472.
67. Baker RR. The generation of formaldehyde in cigarettes – overview and recent experiments. *Food Chem Toxicol*. 2006;44(11):1799-1822.
68. Cooperation Centre for Scientific Research Relative to Tobacco. Determination of Selected Carbonyls in Mainstream Cigarette Smoke by HPLC (No 74). *CORESTA Recommended Method*. 2014;74. Available at: https://www.coresta.org/sites/default/files/technical_documents/main/CRM_74-update%28July14%29.pdf. Accessed January 6, 2018.
69. Gordon DL. PM'S global strategy: Marlboro product technology. A summary developed with input from Batco, Batcof, Souza Cruz, & B&W. Brown & Williamson, Brown & Williamson Tobacco Corporation Research & Development. Available at: <http://www.addictionincorporated.com/wordpress/wp-content/uploads/10-26-92.pdf>. 1992. Accessed January 24, 2016.
70. Spears A. Effect of manufacturing variables on cigarette smoke composition. Brown & Williamson. Available at: <https://www.industrydocumentslibrary.ucsf.edu/tobacco/docs/#id=rrpc0140>. 1974. Accessed January 24, 2016.
71. Newson JR. Evaluation of Competitive Cigarettes. 1975. Available at: <https://industrydocuments.library.ucsf.edu/tobacco/docs/#id=zslf0014>. Accessed February 10, 2016.
72. Seeman JI, Dixon M, Haussmann HJ. Acetaldehyde in mainstream tobacco smoke: formation and occurrence in smoke and bioavailability in the smoker. *Chem Res Toxicol*. 2002;15(11):1331-1350.
73. PMI. What's in Our Products? South Africa. 2012. Available at: <https://www.pmi.com/our-business/about-us/products/how-cigarettes-are-made>. Accessed January 1, 2018.
74. Semchyshyn HM, Miedzobrodzki J, Bayliak MM, et al. Fructose compared with glucose is more a potent glycoxidation agent in vitro, but not under carbohydrate-induced stress in vivo: potential role of antioxidant and antiglycation enzymes. *Carbohydr Res*. 2014;384:61-69.
75. Cocinero EJ, Lesarri A, Ecija P, et al. Free fructose is conformationally locked. *J Am Chem Soc*. 2013;135(7):2845-2852.
76. Takagi Y, Kashiwagi A, Tanaka Y, et al. Significance of fructose-induced protein oxidation and formation of advanced glycation end product. *J Diabetes Complications*. 1995;9(2):87-91.
77. Semchyshyn HM. Reactive carbonyl species in vivo: generation and dual biological effects. *The Scientific World Journal*. 2014;2014:417842.
78. Ahmed N, Furth AJ. Failure of common glycation assays to detect glycation by fructose. *Clin Chem*. 1992;38(7):1301-1303.
79. Baker MDRA. The retention of tobacco smoke constituents in the human respiratory tract. *Inhal Toxicol*. 2006;17:255-294.
80. Roemer E, Wittke S, Trelles Sticken E, et al. The addition of cocoa, glycerol, and saccharose to the tobacco of cigarettes: implications for smoke chemistry, *in vitro* cytotoxicity, mutagenicity and further endpoints. *Beiträge zur Tabakforschung International/Contributions to Tobacco Research*. 2010;24(3):117-138.
81. Coggins CR, Wagner KA, Werley MS, Oldham MJ. A comprehensive evaluation of the toxicology of cigarette ingredients: carbohydrates and natural products. *Inhal Toxicol*. 2011;23(Suppl 1):13-40.
82. Connolly GN, Wayne GD, Lymperis D, Doherty MC. How cigarette additives are used to mask environmental tobacco smoke. *Tob Control*. 2000;9(3):283-291.
83. Wertz MS, Kyriss T, Paranjape S, Glantz SA. The toxic effects of cigarette additives. Philip Morris' project mix reconsidered: an analysis of documents released through litigation. *PLoS Med*. 2011;8(12):e1001145.
84. Canada Tobacco Act. Statutes of Canada. Chapter 27. 2009. Available at: http://www.parl.gc.ca/content/hoc/Bills/402/Government/C-32/C-32_4/C-32_4.PDF. Accessed June 16, 2015.